

Appl. No. 10/575,991
Response Dated June 18, 2009
Reply to Office Action of May 6, 2009

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

Claims 1 to 30 (Cancelled).

31. **(Currently amended)** A composition comprising a protein, wherein the protein comprises onetwo or more redox active amino acids selected from the group consisting of: a 3,4-dihydroxy-L-phenylalanine (DHP), a 3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, a 4-nitro phenylalanine, and a 3-thiol-tyrosine; the composition further comprising:

- i) at least one orthogonal tRNA (O-tRNA), ~~wherein the O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO: 2;~~
- ii) at least one orthogonal aminoacyl-tRNA synthetase (O-RS) comprising a Leu amino acid in a position of the O-RS corresponding to Tyr32 of SEQ ID NO: 4, a Ser amino acid residue in a position of the O-RS corresponding to Ala67 of SEQ ID NO: 4, an Asn amino acid residue in a position of the O-RS corresponding to His70 of SEQ ID NO: 4, or a Gln residue in a position of the O-RS corresponding to Ala167 of SEQ ID NO: 4, and SEQ ID NO: 4 is the wild type sequence; ~~wherein the O-RS comprises at least 90% identity to the RS of SEQ ID NO: 1 or derived from an RS selected from the group consisting of: an *Archaeoglobus fulgidus* synthetase, a *Methanosaecina mazei* synthetase, a~~

~~Methanobacterium thermoautotrophicum synthetase, and a Pyrococcus horikoshii synthetase~~, wherein the O-RS preferentially aminoacylates the O-tRNA with one or more of the redox active amino acids; and
iii) a nucleic acid that encodes the protein, wherein the nucleic acid comprises at least one ~~two~~-selector codon[[s]] that is ~~are~~ recognized by the O-tRNA;
wherein the protein is a product of a translation involving the nucleic acid, the one or
more redox amino acids, the O-tRNA and the O-RS.

32. **(Cancelled)**

33. **(Cancelled)**

34. **(Original)** The composition of claim 31, wherein the composition comprises a pharmaceutically acceptable carrier.

35. **(Currently amended)** The composition of claim 31, wherein the protein further comprises at least one unnatural amino acid that is not a 3,4-dihydroxy-L-phenylalanine (DHP), a 3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, a 4-nitro-phenylalanine, or a 3-thiol-tyrosine.

36. **(Cancelled)**

37. **(Cancelled)**

38. **(Cancelled)**

39. **(Previously presented)** The composition of claim 31, wherein the protein comprises a myoglobin polypeptide or a portion thereof.

40. **(Cancelled)**

41. **(Previously presented)** The composition of claim 31, wherein the protein is capable of undergoing oxidation.

42. **(Previously presented)** The composition of claim 31, wherein the protein is capable of undergoing (i) oxidation or (ii) a shift in reductive peak potential as measured by voltammetric response in comparison to a corresponding protein that lacks said at least two

redox active amino acids, and wherein said oxidation or shift in reductive peak potential require said at least two redox active amino acids.

43. (Currently amended) The composition of claim 31, wherein at least one of said one ~~two~~ or more redox active amino acids is 3,4-dihydroxy-L-phenylalanine (DHP).

44. (Previously presented) The composition of claim 31, wherein the redox active amino acid is a redox catalyst.

45. (Cancelled).

46. (Currently amended) The composition of claim 31, wherein the further comprising ~~an O-RS comprising~~ an amino acid sequence comprising SEQ ID NO: 1.

47. (Currently amended) A composition comprising:

a protein, wherein the protein comprises at least one redox active amino acid selected from the group consisting of: a 3,4-dihydroxy-L-phenylalanine (DHP), a 3,4,5-trihydroxy-L-phenylalanine, a 3-nitro-tyrosine, ~~a 4-nitro-phenylalanine~~, and a 3-thiol-tyrosine,

an O-tRNA,

an aminoacyl-tRNA synthetase (O-RS) comprising an amino acid sequence at least 90% identical to SEQ ID NO: 1 that preferentially aminoacylates the O-tRNA with a redox active amino acid selected from the group, and

a nucleic acid encoding a polypeptide of interest, said nucleic acid comprising at least two selector codons that are recognized by the O-tRNA;

wherein the protein is a product of a translation involving the nucleic acid, the at least one redox amino acid, the O-tRNA and the synthetase.

48. (Previously presented) The composition of claim 47, wherein the synthetase further comprises: a Leu amino acid in a position of the O-RS corresponding to Tyr32 of SEQ ID NO: 4, a Ser amino acid residue in a position of the O-RS corresponding to Ala67 of SEQ ID NO: 4, an Asn amino acid residue in a position of the O-RS corresponding to His70 of SEQ ID NO: 4 or a Gln residue in a position of the O-RS corresponding to Ala167 of SEQ ID NO: 4, and SEQ ID NO: 4 is the wild type sequence.

49. **(Previously presented)** The composition of claim 47, wherein the synthetase further comprises: a Leu amino acid in a position of the O-RS corresponding to Tyr32 of SEQ ID NO: 4, a Ser amino acid residue in a position of the O-RS corresponding to Ala67 of SEQ ID NO: 4, an Asn amino acid residue in a position of the O-RS corresponding to His70 of SEQ ID NO: 4, and a Gln residue in a position of the O-RS corresponding to Ala167 of SEQ ID NO: 4, and SEQ ID NO: 4 is the wild type sequence.

50. **(Cancelled)**

51. **(Currently amended)** The composition of claim 31-50, wherein the synthetase further comprises: a Leu amino acid in a position of the O-RS corresponding to Tyr32 of SEQ ID NO: 4, a Ser amino acid residue in a position of the O-RS corresponding to Ala67 of SEQ ID NO: 4, an Asn amino acid residue in a position of the O-RS corresponding to His70 of SEQ ID NO: 4 or a Gln residue in a position of the O-RS corresponding to Ala167 of SEQ ID NO: 4, and SEQ ID NO: 4 is the wild type sequence.

52. **(Cancelled)**

53. **(Cancelled)**

54. **(Previously presented)** The composition of claim 47, wherein the protein comprises two or more of the redox active amino acids.

55. **(Previously presented)** The composition of claim 47, wherein the synthetase comprises an amino acid sequence at least 95% identical to SEQ ID NO: 1.

56. **(Currently amended)** The composition of claim 31, wherein the further comprising: at least one orthogonal tRNA (O-tRNA), wherein the O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO: 2, or a conservative variant thereof at least 90% identical to SEQ ID NO: 2.

57. **(Currently amended)** The composition of claim 31, wherein the further comprising: at least one orthogonal aminoacyl-tRNA synthetase (O-RS) comprising a synthetase selected from the group consisting of: an *Archaeoglobus fulgidus* synthetase, a *Methanosaicina mazei* synthetase, a *Methanobacterium thermoautotrophicum* synthetase, and a *Pyrococcus horikoshii* synthetase, wherein the O-RS preferentially aminoacylates an O-tRNA with one of the redox active amino acids.

58. **(Previously presented)** The composition of claim 31, wherein the further comprising: a nucleic acid that encodes the protein, wherein the nucleic acid comprises at least two selector codons that are recognized by an O-tRNA.

59. **(New)** The composition of claim 31, wherein the O-RS aminoacylates a reference tRNA of SEQ ID No: 2 with the DHP when the reference tRNA and the DHP are present in the composition.

60. **(New)** The composition of claim 31, wherein the redox amino acid is not incorporated at the protein C-terminus.

61. **(New)** The composition of claim 31, wherein the protein comprises two or more unnatural redox amino acids.